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In vitro antioxidant and free radical scavenging potential of *Amorphophallus paeoniifolius*

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ABSTRACT

The present study is designed to determine the total phenolic compounds, total antioxidant capacity and free radical scavenging properties of methonolic and 70% hydroalcoholic extracts of tubers of *Amorphophallus paeoniifolius* (Dennst) Nicolson (family Araceae). The two *in vitro* models viz. DPPH radical and hydroxyl radical scavenging activity and reducing power assay were carried out to evaluate the antioxidant potential of the extracts. Our results showed that tubers displayed potent antioxidant properties supporting the various ethnomedical uses of drug in various ailments.

Key words: *Amorphophallus paeoniifolius*, antioxidant free radical scavenging, extracts.

INTRODUCTION

The role of free radicals and reactive oxygen species (ROS) in pathogenesis of human diseases and organ toxicities has been widely recognized. Medicinal herbs are known to contain a variety of antioxidants concerned with reactions involving polyphenolic compounds which are reported to posses antioxidant and organ protection properties[1-2]. Hence many herbs / herbal preparations have been adopted in the treatment of various organ toxicities due to xenobiotic / environmental challenges.

Amorphophallus paeoniifolius (Dennst) Nicolson (Araceae) a tuberous, stout indigenous herb commonly known as elephant foot yam, Suran, grown as vegetable is wildly available[3-4] and is reported to contain flavonoids[5]. In Ayurvedic System of Medicine tubers are highly valued in vitiated conditions of Vata, Kapha in treatment of piles, haemophillic conditions, skin diseases, intenstinal warms, obesity, restorative in dyspepsia, debility. The tubers are used as appetizer, tonic and in stomachic[6-9]. The powdered tuber as an ingredient of medicines for cholera & constipation. Plant is a constituent of herbal drug "Leo-cosol-H' which is found to be effective in leucorrohea[10-11]. Tubers are reported in management of haemmorroids[12] to have antiprotease activity[13], antimicrobial activity[14] and analgesic activity of its methanolic extract[5]. However there are no reports on the antioxidant and organ protection profiles of the tubers.

Given the fact of presence of tannins, phenols, flavonoids, triterpenoids and coumarins in methanolic and 70% hydroalcoholic extracts, the present study is aimed to evaluate these extracts for antioxidant activity.

MATERIAL AND METHODS

Plant material

The tubers of Amorphophallus paeoniifolius were collected from cultivated lands from Hassan district of Karnataka and authenticated by Dr.Kotresh, Botany department, Karnataka University, Dharwad.

Extraction

The air dried powder of tubers was subjected to exhaustive soxhlation with solvents Methanol and 70% Hydroalcohol separately. Later the solvent was evaporated on rotary vaccum evaporater below 50°C temperature to get reddish brown methanol extract (ME) and dark brownish 70% hydroalcoholic extract (AE).

Preliminary phytochemical screening

Both the extracts (ME & AE) were screened for the presence of various secondary metabolites, mainly sterols, triterpenoids, flavonoids, tannins, phenols and coumarins using standard method[15-16]. These extracts used for below mentioned experiments.

EXPERIMENTAL

Total phenolic content

To determine total phenolic content working stock solutions of the extracts (ME & AE) were prepared with distilled water to a suitable concentration for analysis. TPC was assessed approximately by using Folin-Ciocalteau Phenol reagent[17].

50µl of extracts (ME : 25mg/ml ; AE : 65mg/ml) were made upto 3ml with distilled water and were added 0.5ml of Folin-Ciocaltean Phenol reagent, incubated for 3 mins at room temperature. Later 2ml of sodium carbonate solution (20% w/v) was added, incubated for 1 minute in boiling water bath. Absorption at 650nm was measured against a reagent blank using shimadza model 150-02 double beam spectrophotometer. The experiment was conducted in triplicate and TPC was expressed as catechol equivalent in milligrams per gram of sample using a standard curve generated with catechol (Fig. 1)

Total Antioxidant Activity

Total antioxidant capacity was measured according to the method previously reported by Pricto[18], with slightly modification. In brief 100 µg of extracts, BHA (Butylated hydroxyl Anisole-as standard) were taken in 0.1ml of alcohol, combined separately in a Eppendroff tube with 1.9ml of reagent solution (0.6ml sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes were caped and incubated in a thermal block at 95°C for 90 minutes. After the samples were cooled to room temperature the absorbance of the aqueous solution of each was measured at 695nm against a blank. A typical blank solution contained 1.9ml of reagent solution and appropriate volume of the sample solvent used for the sample and it was incubated under the same conditions as the rest of the samples. For the samples of unknown composition antioxidant capabilities are expressed as equivalent of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicate and values are expressed as ascorbic acid equivalents in mg per µg of extract (mean ± SD).

Free radical scavenging activity : DPPH radical scavenging activity

The free radical scavenging activity of ME and AE were measured by 1, 1 – diphenyl di-picryl-hydrazil (DPPH) using the method of Singh et al[19] (0.1mM solution of DPPH in methanol was prepared and absorbance was measured at 517nm using shimadza model 150-02 double beam spectrophotometer, referred as absorbance of control reaction). DPPH reagent radily forms free radicals in solution. An antioxidant i.e. free radical scavenger reduces DPPH radical and the extent of violet colour reduction of DPPH is directly proportional to the free radical scavenging activity.

Different concentration (10µg to 500µg) of extracts and BHA as standard in 100 ml methanol were taken and added with 5µL of 0.1mM DPPH in methanol. Shaken vigorously and allowed to stand at 27°C for 20 minutes. Later the absorbance was measured at 517 nm in spectrophotometer using DPPH solution as blank. Lower absorbance of the reaction mixture indicated higher free radical

scavenging activity. Scavenging activity is expressed as the inhibition percentage calculated using the equation: % Antioxidant activity = {(control Abs-Samble Abs)/control Abs} x 100. Each experiment was carried out in triplicate and results averaged, expressed as mean % antiradical activity ± SD.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of ME and AE were studied by using method of Singh et al[19]. To various concentrations of (10 to 25mg) of extracts and BHA made upto 250 ml with 0.1ml phosphate were added with 1ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of EDTA (0.018%) and 1ml of Dimethyl sulphoxide (0.85% v/v in 0.1m phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml ascorbic acid (0.22%) and reaction mixtures were incubated at room temperature for 15 minutes. Later the reaction was terminated by adding 1ml of ice cold TCA (Tricholroacetic acid 17.5% w/v). To all reaction mixtures 3ml of Nash reagent (150 g of ammonium acetate, 5ml of glacial acetic acid and 2ml of acetyl acetone were mixed and raised to 1L with distilled water) was added and left at room temperature for 15 minutes for yellow colour development, the intensity of which was measured spectrophotometrically at 412 nm against reagent blank. The percentage of hydroxyl scavenging activity was calculated by using the equation: 1 – [difference in Abs. of sample / difference in Abs. of blank] x 100. Each experiment was carried out in triplicate and results averaged expressed as mean ± SD.

Reducing ability

Reducing power of ME and AE were determined according to the method of Barreira et al[20] using BHA as standard compound. Various concentration of extracts (10µg-500µg) were mixed with phosphate buffer (2.5 ml, 0.2m, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50^{0}C for 20 minutes. A portion (2.5 ml) of trichloro acetic acid (10% w/v) was added to the mixture which was then contrifused at 3000 rpm for 10 minutes. The upper layer of the solution (5 ml) was mixed with distilled water (5 ml) and $Fecl_3$ solution (1ml, 0.1%) and the absorbance was measured spectrophotometrically at 700 nm. Increased absorbance of reaction mixture indicated increasing reducing power. All the analysis were performed in triplicate and the results were averaged, expressed as mean reducing ability ± SD.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of both the extracts viz. ME and AE showed the presence of sterols, triterpenoids, flavonoids, phenols, tannins, coumarins and carbohydrates.

Total phenolic content (TPC) of ME and AE were 6.16 ± 1.8 mg and 5.14 ± 2.1 mg equivalent to Catechol / gm of extract respectively. As shown in Table 2 the different doses of ME and AE showed antioxidant activities in dose dependant manner in mg equivalent to Ascorbic acid / mg of extract in which ME is nearly potent as BHA (standard).

As phenolics are responsible for antioxidant activity, generally, it is expected that extract / drug which contains high TPC would show highest total antioxidant capacity. But in this case though TPC of ME and AE is less, the total antioxidant capacity is more i.e. nearly matching with standard BHA. This may be due to all the phytoconstituents along with phenolics present in the extracts as stated earlier.

DPPH radical scavenging effect.

DPPH scavenging activity has been used by various researchers as a quick and reliable parameter to asses the *in vitro* antioxidant activity of crude extracts[21]. DPPH reagent radialy forms free radicals in solution. An antioxidant i.e., free radical scavenger reduces DPPH is directly proportional

Table 1: Total phenolic content of Extracts

Extracts	Total phenol content
ME	6.16 ± 1.8
AE	5.14 ± 2.1

Values are in mg equivalent to catechol per gram of extract; values are mean + S.D.

to the free radical scavenging activity. The scavenging activities of DPPH exerted by ME & AE as well as BHA were summarized in Table 3 and Figure 3. ME & AE in a dose dependant manner at the concentrations 500 µg exhibited 42.83% and 72.13% inhibition where as standard compound BHA at concentration 100µg exhibited 79.23% inhibition. And hence in present investigated the AE at higher doses demonstrated good DPPH scavenging activity than ME.

Hydroxyl radical scavenging effect

It is well known that the hydroxyl radical is an extremely reactive free radical formed in biological system causing damage to almost every molecule of living cell. In present study the hydroxyl

Table 2: Total antioxidant capacity* of ME, AE and BHA

Conc. in µg	% of Antioxidant activity of ME µg/mL	% of Antioxidant activity of AE µg/mL	% Anti of BHA µg/mL
10	14 ± 0.13	13 + 0.13	14 + 0.05
25	22 + 0.21	18 + 0.21	23 + 1.32
50	31 + 0.22	28 + 0.21	35 + 0.11
100	52 + 0.44	47 + 1.04	53 + 1.6
200	85 + 0.22	81 + 0.12	90 + 0.23

Conc.: Concentration; * µgs equivalent to ascorbic acid; values are mean + standard deviation

Table 3: Free radical scavenging activity of ME, AE and BHA in DPPH method

Conc. in µg	% Free radical scavenging of ME	% Free radical scavenging of AE	% Free radical scavenging of BHA
10	1.303 ± 0.317	0.773 ± 0.263	14.292 ± 0.291
25	2.600 ± 0.385	5.549 ± 0.455	39.807 ± 0.263
50	10.298 ± 0.525	14.082 ± 0.510	54.056 ± 0.385
100	16.225 ± 0.635	27.280 ± 0.291	79.235 ± 0.263
250	35.141 ± 0.647	60.361 ± 0.193	-
500	42.833 ± 0.569	72.131 ± 0.334	-

Conc.: Concentration; Values are mean ± standard deviation.

Table 4: Hydroxyl radical scavenging activity of ME, AE and BHA

Conc. in µg	% OH radical scavenging of ME	% OH radical scavenging of AE	% OH radical scavenging of BHA
10	5.431 ± 0.693	4.120 ± 0.451	-
25	8.614 ± 0.324	6.133 ± 0.405	3.839 ± 0.649
50	21.067 ± 0.421	6.277 ± 0.693	5.805 ± 0.721
100	34.551 ± 0.243	7.163 ± 0.372	6.742 ± 0.612
250	69.663 ± 0.506	14.794 ± 0.585	9.316 ± 0.633

Conc.: Concentration; Values are mean ± Standard deviation.

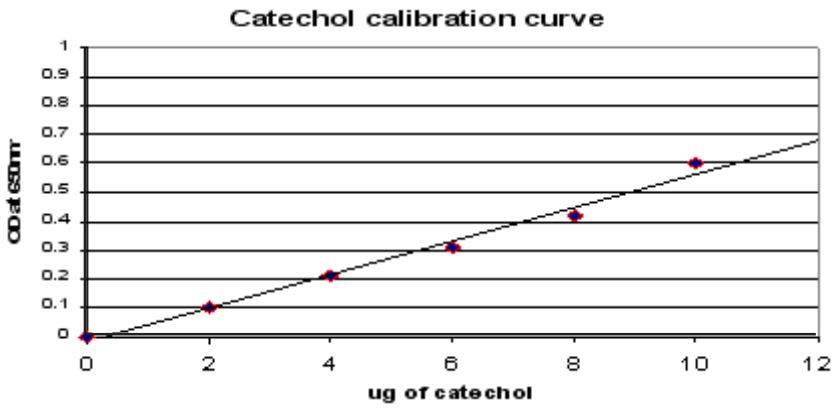

Fig. 1: Catechol calibration curve

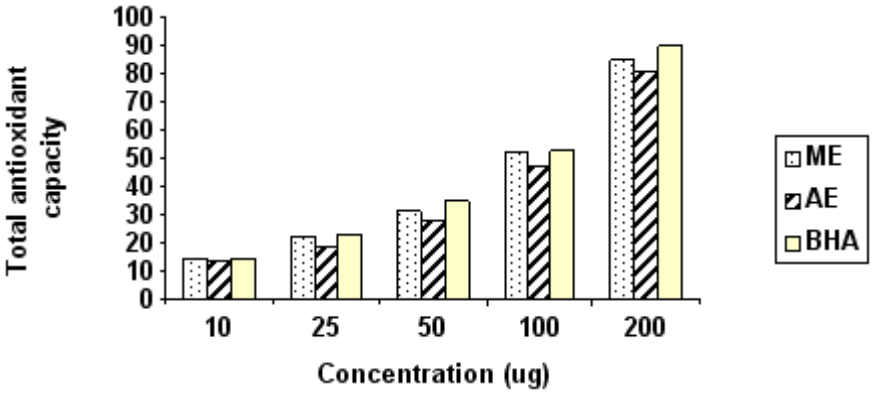

Fig. 2: Total Antioxidant capacity of ME, AE and BHA

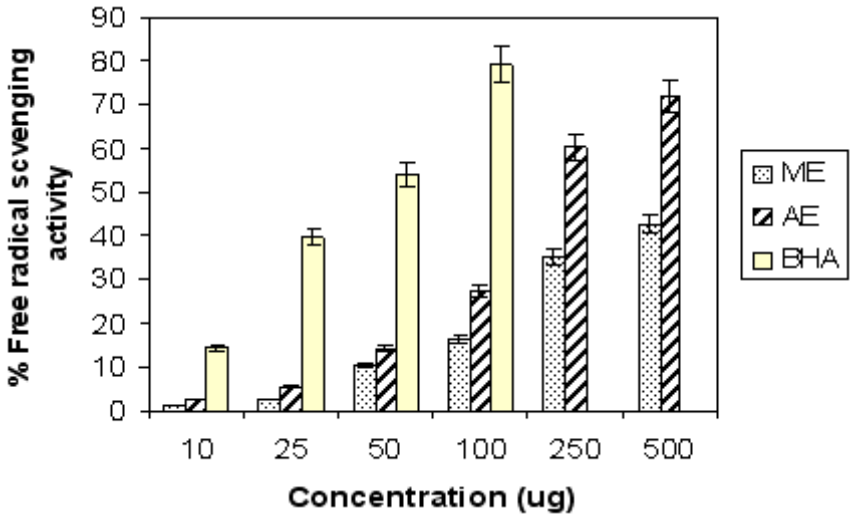

Fig. 3: Percentage inhibition of DPPH radicals by BHA and different doses of ME and AE

radical scavenging effects of ME, AE and standard BHA were summarized in Table 4 and Figure 4. Even though both extracts show dose dependant scavenging activity effect, ME at higher doses of 250µg exhibited 69.663% scavenging effect, AE and BHA exhibited 14.794% and 9.316% scavenging respectively, indicating ME is more potent than AE and BHA.

Reductive ability

The antioxidant activity has been reported to be concomitant with the development of reducing power[22]. The reducing power of the extract might be due to its hydrogen donating ability, reducing Fe^{3+} and converting it to Fe^{+2}. The comparative reducing power of ME, AE and BHA is shown in Table 5 & Figure 5. The degree of absorbance of reaction mixtures indicated the reduction potential of extracts. All the amounts of ME and AE showed good activity than control and demonstrated dose dependant increase in the reducing property where in 500µg of both ME & AE have shown nearly equivalent reduction ability of 50µg of standard BHA.

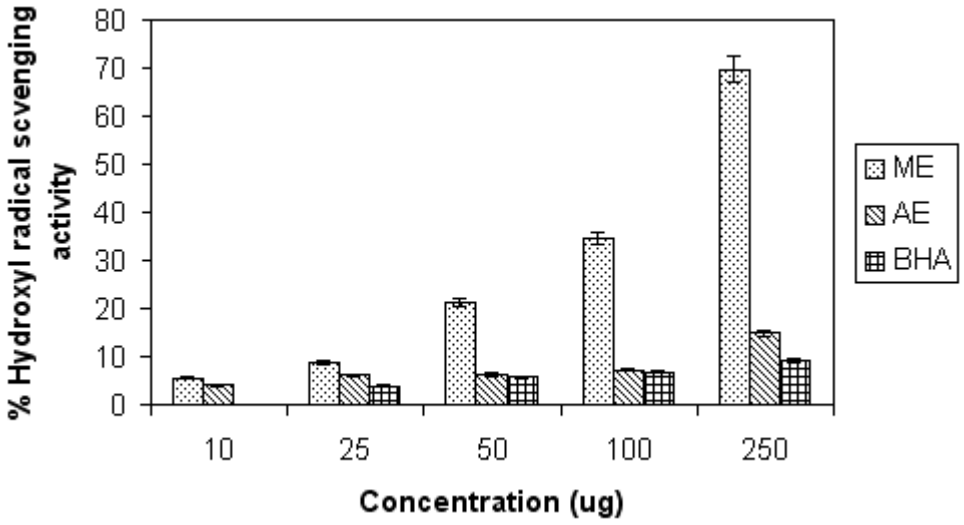

Fig. 4: Percentage inhibition of hydroxyl radicals by BHA and different doses of ME and AE

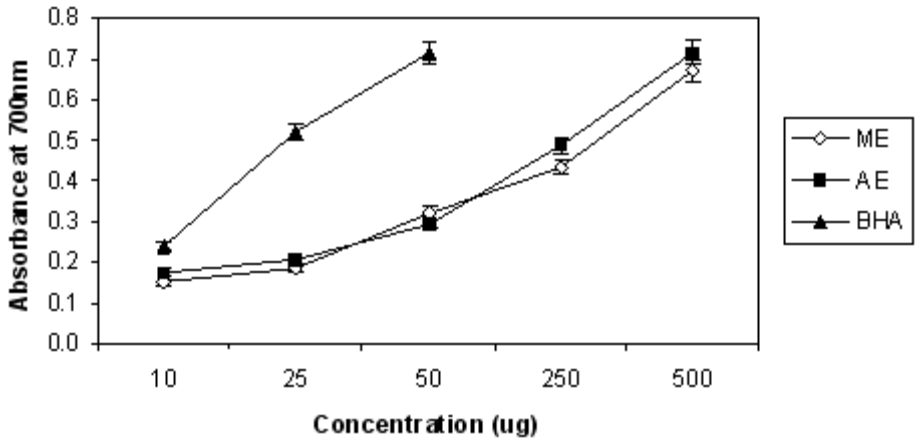

Fig. 5: Reducing ability of BHA and different doses of ME and AE

Table 5: Reducing power assay* of ME, AE and BHA

Conc. in µg	Reducing ability of ME	Reducing ability of AE	Reducing ability of BHA
10	1.149 ± 0.004	0.176 ± 0.002	0.237 ± 0.004
25	1.82 ± 0.003	0.203 ± 0.003	0.52 ± 0.002
50	0.32 ± 0.003	0.294 ± 0.004	0.713 ± 0.003
250	0.433 ± 0.002	0.484 ± 0.004	-
500	0.671 ± 0.005	0.716 ± 0.004	-

Conc.: Concentration; * Absorbance at 700nm, Values are mean ± standard deviation

CONCLUSION

Thus, the present study may be concluded that both Methonolic and 70%Hydroalcoholic extracts posses antioxidant properties which may be attributed to the antioxidant principles including flavonoids, phenols, tannins coumarins etc. present in them. Further studies are in progress to evaluate the *in vivo* antioxidant potential of the extracts in various animal models to establish organ protective properties of the drug.

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